MITOTIC ACTIVITY OF WOUNDED HUMAN EPIDERMIS*

DENNIS J. SULLIVAN, B.S. AND WILLIAM L. EPSTEIN, M.D.

This study deals with the mitotic response of wounded human epidermis. We will demonstrate that mitoses appear in several distinct and transient bursts rather than throughout a single period of high activity as previously thought (1–5).

MATERIALS AND METHODS

We made seventeen to twenty-five wounds, 5 mm. long and 2–4 mm. deep, with a razor blade on the lateral aspect of the lower legs of each of four healthy white males, twenty-five to thirty-five years old. During the ensuing four days, biopsy specimens were obtained at precisely six hour intervals with an electric rotary punch. Each wound was examined by biopsy once. Six hours before biopsy specimen removal 0.3 mg. Colcemid (n-desacetyl-methyl colchicine†), which is less toxic than colchicine (6) was injected intradermally at the wound site to arrest mitotic activity. The biopsy specimens were fixed immediately in 10 per cent neutral formalin, embedded in paraffin, sectioned at 7 μ and stained with hematoxylin and eosin. Three additional healthy white males were tested in the same way except that the full complement of biopsy specimens was not obtained.

Mitotic activity was estimated by counting the number of dividing cells seen in 4000 intact nuclei within four highpower (400 ×) fields on each side of the wound. One difficulty was to decide which cells were capable of dividing. The disruption in the epidermis near the edge of the wound so distorted the usual relationships of epidermal cells as to make positive identification of cell layers impossible. Since we occasionally saw mitoses directly beneath the granular layer we included in our counts all intact nuclei below this layer (Figs. 1 and 2). Bullough has made similar observations (7). We ignored prophase and the rare anaphase or telophase figures in our counts and recorded only metaphase, to avoid subjective error in identifying less obvious mitotic figures. Eight sections selected at random from each specimen were examined. The approximate mitotic index was expressed as dividing nuclei per 1000 interphase nuclei.

This approximation of a mitotic index is necessarily quase-quantitative. In order to arrive at a strictly quantitative analysis one must know precisely which cells are capable of dividing, but no method has yet been devised to establish this fiducial point. Another difficulty arises from our unprecedented use of intradermal Colcemid. Hooper (8) has demonstrated that the systemic administration of colchicine completely arrests mitosis in the gut for periods up to 3 hours without exerting cytotoxic effects. We allowed the drug to act for 6 hours, but cannot be certain that all mitoses were arrested, even though figures in anaphase and telophase were seen rarely. Local cytotoxic effects, however, remained minimal.

RESULTS

The results obtained are tabulated in Figure 3. The use of an agent that arrests metaphase allowed us to demonstrate that healing of incisional wounds, as in other forms of skin injury (1–5), is accompanied by bursts of mitotic activity. Most divisions occurred at the edge of the wound, with a decreasing gradient of mitoses farther from the edge. The level of mitotic activity 2 mm. away from the wound invariably approximated that seen in uninjured skin. Very often mitotic figures occurred in groups of 2 or 3; this phenomenon, first described by Flemming (9) in 1884, remains unexplained. In addition, we observed two or three transient bursts of mitotic activity that lasted 6 to 10 hours (Fig. 3). Each peak was followed by 12 to 18 hours of low or "normal" activity. Although the onset of high mitotic activity varied with the individual, it usually began during the third day after wounding. The initial mitotic burst did not correlate with epithelial closure. In three subjects, the first period of cell division preceded epithelial closure and in the fourth it followed closure. In the three additional subjects in whom the study was not completed we were able to follow the pattern of response through the first period of increased cell division. The mean time of onset of activity in all seven subjects was 55 plus

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* From the Division of Dermatology, Department of Medicine, University of California School of Medicine, San Francisco, California.

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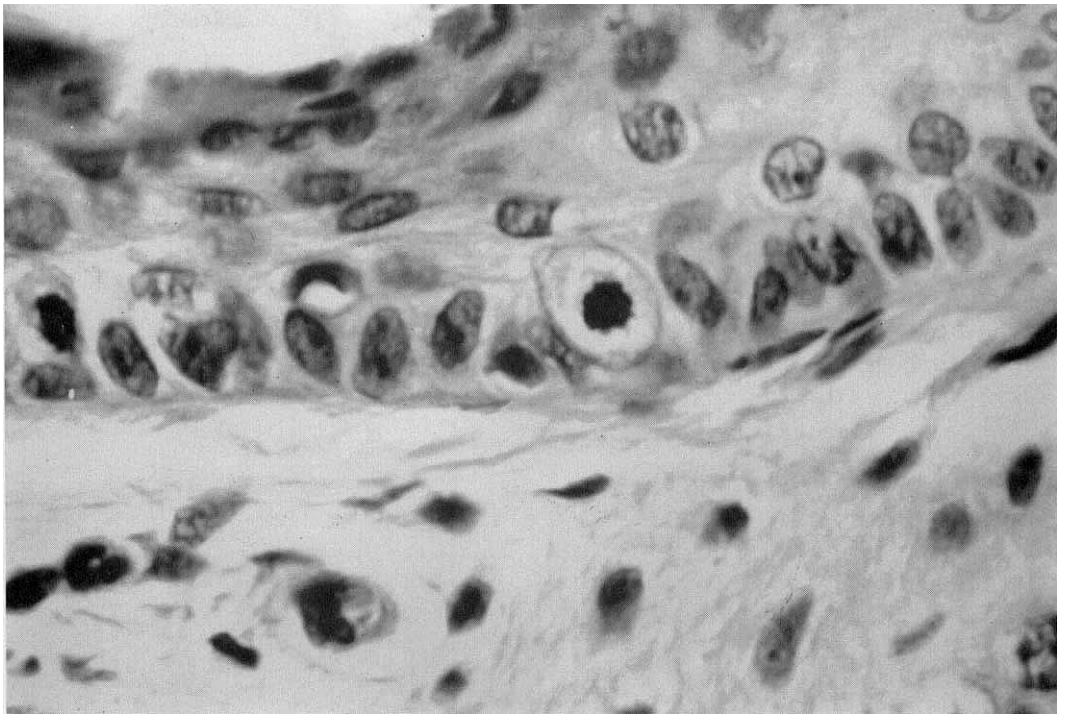

FIG. 1. A cell of the external root sheath arrested in metaphase by Colcemid. The cytoplasm is swollen, the nuclear membrane absent, and the nuclear chromatin condensed and irregular. This is a typical "colchicine cell."

or minus 13 hours. This spread compares favorably with the finding that after skin stripping the onset of mitotic activity varies from 42 hours (4) to 72 hours (10).

Intradermal injections of Colcemid in total doses up to 2.4 mg. in 24 hours produced no evidence of systemic toxicity. It caused some acute inflammation locally, but this did not seem to interfere with the mitosis-arresting effect or with wound healing. Intradermal injection of Colcemid in unwounded skin blocked no more than 2–3 mitoses/thousand.

The periodic acid-Schiff reaction controlled with diastase showed glycogen in epidermal cells far beyond the area of mitotic activity. In one instance, glycogen was seen in epidermal cells 4 to 5 mm. away from the wound margin. This lends support to Lobitz's observation that glycogen accumulation does not necessarily connote impending cell division (11).

DISCUSSION

The mitotic counts we report here are not strictly comparable to those found by other investigators. In studying the responses of human skin to injury, others have employed cellophane tape stripping without addition of mitosis-arresting agents (1–5), and in their mouse skin slicing experiments, Bullough and Laurence (12) incubated the biopsy specimens with colchicine. We produced small razor slices and arrested mitoses for six hours with intradermal Colcemid. Our experimental procedure, in addition to facilitating the counting of mitotic figures, allowed us to observe the full pattern of cell division throughout a four day period.

Early investigators, unable to see increased mitoses at the margin of wounds, disagreed as to whether mitosis (13, 14) or amitosis (15, 16) was responsible for re-epithelialization. We no longer question the fact that only mitosis occurs (1–5, 7, 11, 12, 17, 18). Contrary to the commonly held view that after injury, epidermal mitoses rapidly increase and remain at a high level until healing is complete, our data reveal several transient bursts of mitotic activity. The periods of high mitotic response come in waves and appear as sharp peaks against a background of

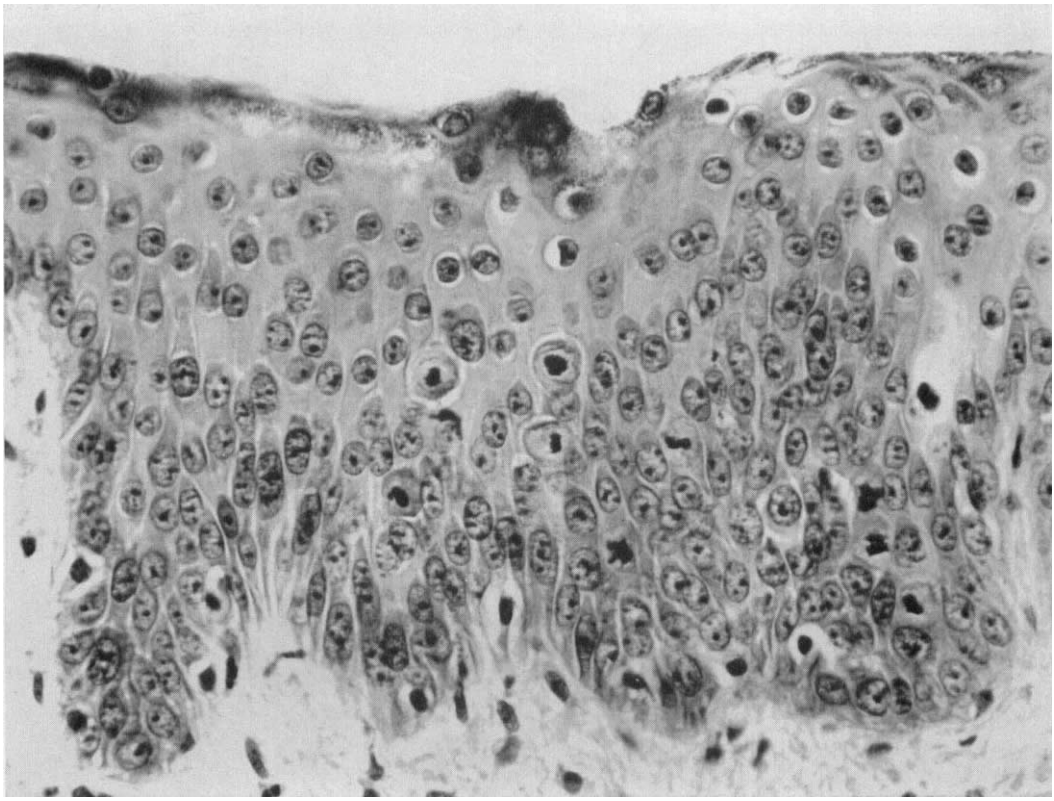

FIG. 2. The epidermis near the edge of a wound during a burst of mitotic activity. The disturbed cell morphology makes it difficult to identify the normal strata. Examination of serial sections reveals mitoses at all levels below the granular layer.

"normal" activity. These waves bear no relation to epithelial closure. (This is in contrast with the findings in mice, in which epithelialization always precedes the first mitotic burst (12)) Although the time of onset of high mitotic activity varies with each individual, the intervals between bursts remain relatively constant in a given subject. Such a pattern of response suggests the presence of parasynchronous cell division (19). If this can be proved with certainty, it should be possible to calculate mathematically the duration of mitosis and the regeneration time (20, 21).

It should be emphasized that the cyclic pattern of mitotic activity seen in this study is distinct from other recognized patterns of cyclical cell division in skin: 1) In *diurnal cycles*, the frequency of mitosis is slightly higher during the periods when the animal rests than during periods when it is active (18, 22, 23). Current evidence suggests that release of adrenalin may control this cycle (24). 2) Variations occur during the *estrus cycle* (25). 3) There are changes correlated with *hair growth cycles* (18, 26). 4) Also, it seems that cyclical alterations occur with *aging*. In young animals the epidermis has a high mitotic rate that becomes lower with maturity, increases again with middle age, and then sinks back to low levels with senility (18).

Observations of the enhanced mitotic rate during wound healing have given rise to speculations concerning mechanisms that control it. Some have suggested the occurrence of a hypothetical "wound hormone" that stimulates mitotic activity (27). Bullough (7, 17), who challenges this, views cells as being ever ready to divide but held in check by some local inhibitor. When the alleged inhibitor is removed or neutralized, as after wounding, the mitotic rate promptly rises until the inhibitor is restored. This concept is not easily reconciled with the periodic bursts of mitotic activity we see after wounding human skin. We wonder if substrate utilization might not act as the stimulus for cell division in wound healing. During the time of rapid

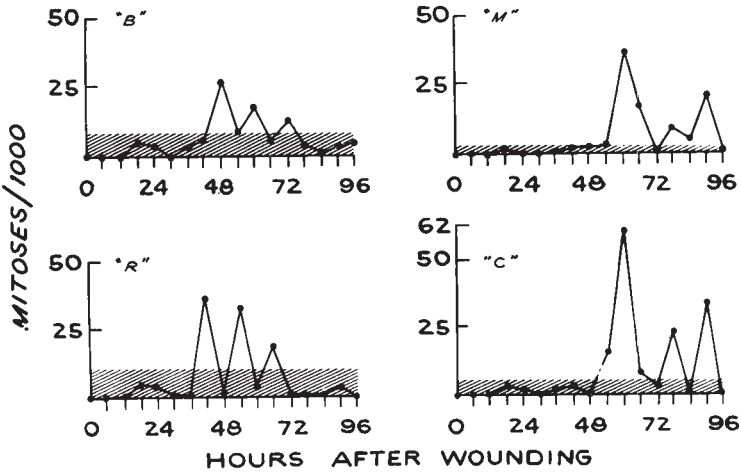

FIG. 3. The epidermal mitotic response to wounding in four subjects. Several distinct bursts of mitotic activity are seen to occur; each is separated by a period of "normal" or low activity. The cross-hatched areas represent a level 5 times the mean level of mitotic activity for the first 36 hours.

formation of nucleoproteins one can anticipate periods when a needed substrate is completely utilized and the trigger for mitosis is released. The *in vitro* observations of Xeros (28) give support to this notion; thymidine added to cultures of Chang-appendix cells inhibited cell division. When thymidine was washed out, a burst of parasynchronous cell division occurred within 5 to 10 hours. It remains to be learned whether a depletion of thymidine or nucleosides occurs *in vivo*.

SUMMARY

1. The mitotic response of epidermal cells was determined during surgical wound healing in human volunteers. Injury was produced by razor slices; mitoses were arrested by intradermal injections of Colcemid; and biopsy specimens were obtained every six hours for 4 days.

2. Each subject showed, not one, but two or three transient bursts of high mitotic activity, followed by periods of "normal" mitotic response. The first mitotic burst began between 42 and 60 hours; the mean time of onset was 55 ± 13 hours. The other bursts followed regularly at 12 to 18 hour intervals.

3. The significance of these findings in relationship to the present concepts of mitotic rate during wound healing is discussed.

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